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DO FISH REPEL OVIPOSITION BY *AEDES TAENIORHYNCHUS*?

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ABSTRACT. We tested the hypothesis that fish indirectly control mosquitoes by repelling oviposition. *Aedes taeniorhynchus* oviposition in mangrove forests, natural pools, and excavated pools with different concentrations of fish was compared. Oviposition in adjacent mangrove forests was concentrated in the site with the lowest fish density. In 3 mangrove forests, egg populations from pool banks were significantly ($P < 0.001$) lower for pools with fish than for fishless pools, with an overall mean ± SD of 1.6 ± 5.7 and 20.0 ± 34.8 eggs/sample, respectively. At one pool, egg densities increased significantly ($P < 0.001$) from 0.36 to 5.00 eggs/sample after drying killed fish. In excavated pools, oviposition was significantly ($P < 0.001$) affected by fish. These data indicate that *Ae. taeniorhynchus* avoids ovipositing in sites with high concentrations of fish and suggest that the response is fish-mediated. This behavior may help to explain the migratory habits and population dynamics of *Ae. taeniorhynchus.*

INTRODUCTION

Although fish are frequently used in mosquito biological control programs (Service 1983), the mechanism of control is rarely defined. In addition to direct predation of mosquito larvae, aquatic predators may have significant indirect effects on mosquitoes. Mosquitoes may selectively avoid ovipositing in water with notonectids (Chesson 1984), fish, and tadpoles (Petranka and Fakhoury 1991). Controlled experiments with the mosquito *Anopheles punctipennis* (Say) established that fish detection was chemically rather than visually mediated (Petranka and Fakhoury 1991).

Fish appear to play an integral role in the population dynamics of the black salt marsh mosquito (*Aedes taeniorhynchus* Wied.). Population crashes have been associated with high water levels, with potential mechanisms including increased populations of larvivores, submergence of mosquito oviposition sites, and death of unhatched, submerged eggs (Ritchie 1984). Additionally, recent studies suggest that fish may influence oviposition. *Aedes taeniorhynchus* oviposits on exposed salt marsh and mangrove soil and is influenced by a variety of environmental factors (for a review see Ritchie and Addison [1992]). Eggshell studies indicate that oviposition is concentrated in rain-flooded mangrove basin forests with, presumably, lower populations of fish than tidally flooded sites (Ritchie and Addison 1992). We tested the hypothesis that *Ae. taeniorhynchus* avoid ovipositing in mangrove swamps and pools containing high densities of fish. Furthermore, a controlled field experiment provides preliminary evidence that fish mediate this response.

MATERIALS AND METHODS

Oviposition in adjacent mangrove forests with high and low populations of fish: Water conditions, fish populations, and associated *Ae. taeniorhynchus* oviposition in 2 adjacent (separated by a 10-m-wide berm) mangrove basin forests (April Court and Dogwood) in Collier County, FL, are described. These sites are respectively described by Ritchie and Johnson (1991a, 1991b). Mosquito oviposition was estimated by egg sampling (data derived from Ritchie and Johnson 1991a, 1991b) or by dipping for larvae. Because tides may move larvae from the oviposition site, only broods hatched by rainfall are presented. Hatching rates approaching 100% (Ritchie and Johnson 1991b) indicate that most eggs collected from recently exposed soil were laid during the period since soil had been exposed (Ritchie and Johnson 1991a). Aquatic predators were sampled biweekly at each site with 2 modified Gee's® minnow traps (Ritchie and Johnson 1986) set for 24 h. Water salinity was measured with a refractometer. The percent submergence was estimated from a water depth–cumulative area function (Ritchie 1988[2]) based on 200 elevation points (measured with a level and transit) within each site. Water depth was measured at a reference staff gauge or recording water table well.

Oviposition at natural pools with and without fish: Unique conditions allowed us to compare

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Ae. taeniorhynchus oviposition in fish and fishless pools within the same site. In 1992, mangrove forests were submerged for most of the winter, with tidal flooding in February supplementing existing fish populations. A drought in April–May dried many mangrove forests, isolating fish in the larger pools. Pool banks, a preferred oviposition site (Ritchie and Addison 1992), are nearly devoid of eggs following prolonged submergence (S. A. Ritchie, unpublished data). Oviposition on exposed banks resumes from emigrants in spring. Thus we could test the hypothesis that *Ae. taeniorhynchus* avoids ovipositing at fish pools by comparing pool bank egg populations from pools with and without fish.

Aedes taeniorhynchus eggs were sampled from the exposed banks of flooded pools (fish pools) and recently dried pools (fishless pools) in 3 black mangrove (*Avicennia germinans* Linn.) basin forests. Study sites at Marco Island, Key Island, and Rookery Bay in Collier County, FL, were sampled in late May 1992. Drying out of a large fish pool at Marco Island on May 23 allowed us to compare egg populations at the same pool both with and without fish. Sampling transects (6 per site) were randomly selected along sections of pool banks with moderate slope and detrital cover, known attributes of *Ae. taeniorhynchus* oviposition sites (Ritchie and Addison 1992). A 12-cm-diam polyvinylchloride pipe corer was used to take 3 and 2 respective soil cores at equidistant intervals below and above the area where black mangrove pneumatophores appeared in each transect. Because the pneumatophore threshold approximates a plane of equal elevation within each forest, relative elevation and thus soil moisture content were standardized. Each soil core was placed in a ziplock plastic bag, incubated for 72 h then flooded with a dilute yeast solution to hatch eggs. To test if the absence of eggs in fish pools was due to inhibited egg hatch, soil samples from one fish pool (Marco Island) were then processed for eggs by sieving and bleaching (Ritchie and Johnson 1989) and eggs examined for embryonation after clearing in 5% sodium hypochlorite.

Fish were sampled using the trap described earlier. Fish collections are summarized as percent poeciliids (*Gambusia holbrooki* [Girard], *Poecilia latipinna* [Lesueur]); all remaining fish were cyprinodontids (*Rivulus marmoratus* [Poey], *Fundulus confluentus* Goode and Bean, and *Cyprinodon variegatus* [Lacepede]). All are known larvivores (Harrington and Harrington 1961). Poeciliids are live bearers that are introduced from other sites by tides or runoff (Harrington and Harrington 1961) whereas cyprinodontids can survive as eggs and adults in a dry swamp (Taylor et al. 1992). Mosquito egg counts in fish and fishless pools were transformed by $\log(x + 1)$ and compared using an unpaired t-test.

Oviposition in excavated pools with and without fish: Excavated pools were used to examine *Ae. taeniorhynchus* oviposition in response to fish under controlled conditions in a black mangrove forest. Pools (45 cm wide × 40 cm deep, volume about 40 liters) were excavated by shovel in 2 parallel rows of about 15 cm elevational difference on April 9, 1992. Each row contained 6 pools 1–2 m apart. A 45–60° slope was constructed on the upslope side and covered with a layer of newly fallen leaves to provide shelter for mosquitoes without adding eggs. To estimate the number of resident eggs present in pool soil before exposure to mosquitoes, 1.1 liters of soil was collected from pool banks and processed for eggs by sieving and bleaching (Ritchie and Johnson 1989). Pupal water, a suspected oviposition attractant (Ikeshoji and Mulla 1970), was collected from the site during a mosquito emergence and 1.2 liters added to each pool. The water salinity was 25 ppt. Because disturbed salt marsh can emit hydrogen sulfide, a fish toxicant, we waited 18 h before adding 15 *G. holbrooki* to 3 randomly selected pools in each row. The mean ± SD standard length for a subsample of 30 fish was 31 ± 8 mm.

Partial flooding of pools on April 19 necessitated sampling the next day. Although some dead fish were noted, live fish were observed in all but the control pools. Soil was excavated from the sides and bottom of each pool using a mason's trowel and a fillet knife to cut roots; about 3 liters of soil was taken from each pool. Soil was processed for eggs and newly hatched eggshells by sieving and bleaching (Ritchie and Johnson 1989); because rain may have hatched some eggs, newly hatched eggshells were tallied as eggs. This experiment was repeated again twice.

RESULTS

Oviposition in adjacent mangrove forests with high and low populations of fish: During the sampling periods, *Ae. taeniorhynchus* oviposited at Dogwood despite high water levels, low salinity, and low (< 2 fish/trap with 44% poeciliids) fish populations (Table 1). High egg populations in 1987 indicate that oviposition is not restricted to low salinity conditions. In contrast, oviposition at April Court was restricted to periods of relatively low (1987) or no (August 1986) fish populations (Table 1). Fish populations at April Court consisted of 38 and 20% poeciliids during the 1986 and 1987 sampling period, respectively.

Oviposition at natural pools with and without fish: A total of 198 and 2,394 eggs were collected from fish and fishless pools, respectively, with

Table 1. *Aedes taeniohynchus* oviposition,[1] water quality, and fish populations in adjacent mangrove forests.

Dogwood					April Court				
Oviposition window	Submergence (%)	Salinity (ppt)	Fish per trap[2]	Oviposition	Oviposition window	Submergence (%)	Salinity (ppt)	Fish per trap[2]	Oviposition
July 9–17, 1986	96 ± 3	2 ± 1	0.2 ± 2[3]	12.7 ± 17.5	July 24–31, 1986	54 ± 35	22 ± 9	56.7 ± 55.6[4]	0
July 18–Aug. 12, 1986	72 ± 16	5 ± 2	1.3 ± 1.2[3]	6.9 ± 16.3	Aug. 1–12, 1986	site dry	site dry	site dry	1.4 ± 2.8[5]
Jan. 3–Mar. 4, 1987	58 ± 29	16 ± 8	0.7 ± 0.6[3]	15.6 ± 29.8	July 14–28, 1987	72 ± 31	22 ± 4	9.0 ± 7.0[4]	3.6 ± 9.0

[1] Oviposition is the mean (±SD) number of eggs/12-cm-diam soil core (see text).
[2] Mean ± SD number of fish/trap/day.
[3] Also collected a mean of <5 insects and hylid tadpoles/trap.
[4] Also collected no insects or tadpoles and a mean of <2 crabs and <6 shrimp/trap.
[5] Mean number of 1st-instar larvae per dip (n = 50) collected 18 h after flooding rain on August 12.

highly significant ($P < 0.001$) differences at all sites (Table 2). Mean (± SD) egg density increased significantly ($t = 3.53$, df = 58, $P < 0.001$) from 0.36 ± 1.16 to 5.00 ± 9.02 eggs/sample after drying of the Marco Island fish pool killed the fish. Fish pools also contained large numbers of aquatic insects (Table 2), all dytiscid and hydrophilid beetles. None of the 15 eggs recovered by sieving and bleaching soil cores from a fish pool were fertile (13 were infertile and 2 had deformed embryos).

Oviposition in excavated pools with and without fish: Oviposition was significantly affected by fish ($F = 62.4$; df = 2,9; $P < 0.001$), elevation ($F = 44.3$; df = 2,9; $P < 0.001$) and their interaction ($F = 6.1$; df = 2,9; $P = 0.04$) in the first excavation experiment. The number of eggs was significantly ($P < 0.01$) lower in fish pools than in fishless pools for high and low elevations (Fig. 1). Accounting for resident eggs (estimated to be 40/pool), little if any oviposition took place in pools with fish. In the remaining 2 trials, fish escaped after heavy rain flooded the pools.

DISCUSSION

Aedes taeniorhynchus avoided ovipositing near water with a high concentration of fish. This was observed both within a site (natural pool studies) and between sites (oviposition at Dogwood and April Court in late July 1986) with divergent concentrations of fish. Other factors do not appear to account for the observed oviposition patterns. Avoidance of flooded or large pools does not explain why oviposition occurred at Dogwood despite standing water nor why oviposition at April Court and the Marco Island fish pool commenced after the sites dried. Similarly, the onset of oviposition upon the drying of fish pools with no recent history of mosquito production is not consistent with selective oviposition in response to a pupal pheromone (Ikeshoji and Mulla 1970), although decomposing fish could be an oviposition attractant. Elevation, soil sulfates, salinity, and moisture, known to affect oviposition (Knight and Baker 1962, McGaughey 1968, Ritchie and Johnson 1991b), were controlled in the excavation experiment and, presumably, in the natural pool study where a standardized elevation and a common water table ensured comparable soil conditions. High concentrations of insect larvivores, such as dytiscids, were found only in 2 of the sites in the natural pool study. Only high concentrations of fish were consistently associated with low egg populations.

Selection pressure for oviposition in low density fish sites would be strong. A *Gambusia* population of 30/m² (equivalent to 100/trap based on formula from Stewart and Miura [1985]), each

Table 2. *Aedes taeniohynchus* egg densities[1] at natural pools with and without fish in 3 black mangrove forests in southwestern Florida.

Site (date sampled in 1992)	Pools without fish		Pools with fish		
	Pool sizes (m^2)	Mosquito eggs	Pool sizes (m^2)	Fish/trap[2,3]	Mosquito eggs
Marco Island (May 19)	35, 20	27.6 ± 42.1A	600	204.5 (96%)	0.36 ± 1.16B
Key Island (May 26)	21, 30	31.6 ± 44.9A	330, 110	26.5 (87%)	3.12 ± 7.78B
Rookery Bay (May 29)	25, 30	15.7 ± 25.6A	250, 90	109.0 (95%)	0B

[1] Mean ± SD number of eggs hatched from 30 12-cm-diam soil cores per pool; means in the same row followed by a different letter are significantly different ($P < 0.001$) by unpaired t-test on log(x + 1) transformed egg counts.

[2] Number of fish collected in a modified Gee's Improved Minnow Trap® over a 24-h period with percent poeciliid fish (all others cyprinodontids).

[3] Also collected a respective mean of 75, 37, and 10 aquatic beetles/trap at Marco Island, Key Island, and Rookery Bay.

capable of eating 550 *Aedes* larvae during the course of a brood (Ritchie and Montague, in press), could potentially consume 17,000 larvae/m^2 (about 120 larvae/dip). With almost certain predation facing larvae hatched near fish ponds, a strategy of not ovipositing in such habitat could evolve.

Such a strategy might explain the biology of *Ae. taeniorhynchus*. The spectacular migrations of recently emerged females (Provost 1957) may ultimately take females to dry, fishless oviposition sites. Fish-mediated oviposition repellency may, in part, account for the high oviposition rates in rain-flooded sites (Ritchie and Johnson 1991b, Ritchie and Addison 1992); low fish densities in these sites (e.g., Dogwood) are associated with nearly continuous mosquito oviposition. Finally, this strategy suggests how prolonged wet weather results in minimal mosquito populations (Ritchie 1984). Fish-free oviposition sites become increasingly rare as rising water tables spread fish. The increased searching time for isolated oviposition sites, over-crowding of larvae from aggregated oviposition, and the dilution of adults emigrating from scattered breeding sites might all contribute to reduced populations of biting females. Submergence of oviposition sites, death of flooded unhatched eggs and larval predation may also contribute to low mosquito populations (Ritchie 1984).

Despite the association of oviposition with fishless sites, the controlled field experiment provides only preliminary evidence that the behavior is fish-mediated. Because *Gambusia* can significantly affect pond biota and water quality (Hurlbert et al. 1972), an array of cues to detect fish would be available. Detection mechanisms also vary and include visual, olfactory, and contact chemoreception (Petranka and Fakhoury 1991). Our data suggest the cue acts over a short distance and lasts but a few days. Oviposition occurred in fishless pools within 5 m of fish pools and resumed in fish pools shortly after they dried.

Additional experiments are needed to verify and identify the nature of a fish-mediated ovipositional repellent. Laboratory experiments, although convenient, may misrepresent the array of cues and behaviors observed in the field. Artificially constructed pools may repel ovipositing mosquitoes; use of natural or "aged" pools might improve oviposition. Increasing the exposure time would allow eggs to accumulate but invites the disaster of flooding and the loss of all data; many replicates may be needed to get an acceptable trial. Use of caged fish would control for visual cues (Petranka and Fakhoury 1991)

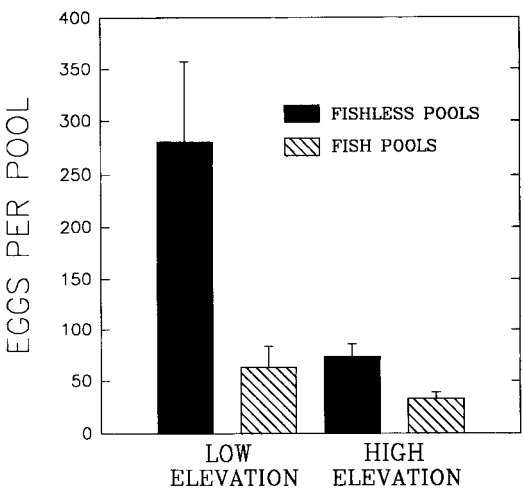

Fig. 1. Mean number (+SD) of *Aedes taeniorhynchus* eggs from excavated pools with and without fish for low and high elevational strata; egg numbers were significantly different ($P < 0.01$) by an unpaired t-test for both elevations.

and might permit eggs to be hatched *in situ,* eliminating tedious soil sampling and sieving.

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